

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 9/127</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/30697</b> <b>(43) International Publication Date:</b> 28 August 1997 (28.08.97)
<b>(21) International Application Number:</b> PCT/US97/02385 <b>(22) International Filing Date:</b> 18 February 1997 (18.02.97) <b>(30) Priority Data:</b> 08/604,448                      21 February 1996 (21.02.96)                      US <b>(60) Parent Application or Grant</b> (63) Related by Continuation US    08/604,448 (CON) Filed on                                      21 February 1996 (21.02.96) <b>(71) Applicant (for all designated States except US):</b> NORTH CAROLINA STATE UNIVERSITY [US/US]; 103 Holladay Hall, Campus Box 7003, Raleigh, NC 27695-7003 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SMART, Robert, C. [US/US]; 1324 Mayfair Road, Raleigh, NC 27607 (US). OH, Hye-Sun [KR/US]; P-203 E.S. King Village, Raleigh, NC 27607 (US). <b>(74) Agents:</b> SIBLEY, Kenneth, D. et al.; Bell, Seltzer, Park & Gibson, P.O. Drawer 34009, Charlotte, NC 28234 (US).		<b>(81) Designated States:</b> AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> METHOD OF TREATING ALOPECIA  <b>(57) Abstract</b>  A method of enhancing hair growth or treating alopecia in a subject uses topically administered estrogen receptor antagonists. Pharmaceutical formulations comprising estrogen receptor antagonists are described.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

## METHOD OF TREATING ALOPECIA

This invention was made with Government support under grant CA46637 from the National Cancer Institute and grant ES07046 from the National Institute for  
5 Environmental Health Sciences. The Government has certain rights to this invention.

### Field of the Invention

This invention relates to methods of treating hair loss and promoting hair growth, and more  
10 particularly to a method of using estrogen receptor antagonists to promote hair growth in a subject in need of such treatment.

### Background of the Invention

Alopecia (baldness) is a deficiency of hair,  
15 either normal or abnormal, and is primarily a cosmetic problem in humans, although the negative psychological impact of hair loss is well known. See C.H. Mortimer et al., *Clin. Exp. Dermatol.* 9, 342-350 (1984). Dermatologists recognize many different types of  
20 alopecia, with androgenic alopecia being the most common cause of hair loss in both men and women. As this type of hair loss is more common and more severe in males, it is typically referred to as "male pattern baldness." However, it is thought that androgenic alopecia affects  
25 more than one third of individuals of either sex who have a strong family history of hair loss. See W.F. Bergfield, *Clin. Dermatol.* 6, 102-107 (1988).

One traditional treatment for alopecia is the method of hair transplantation. Typically, this method  
30 involves transplanting plugs of natural hair from areas of the scalp where hair is growing to bald areas. This

-2-

procedure is costly, time-consuming, painful, and meets with only limited success.

Another common treatment for hair loss is the application of a chemical or drug for the purpose of stimulating hair growth. For example, U.S. Patent No. 5,177,061 to Pickart proposes the topical application of glycyl-L-histidyl-L-lysine:copper(II) (GHL-Cu) and its derivatives to promote hair growth in warm-blooded animals (applicant specifically intends the disclosure of this and all other patent references cited herein to be incorporated herein by reference in their entirety). U.S. Patent No. 4,832,946 to Green proposes a composition for topical application to mammalian hair or skin, comprising an amount of the cell-free supernatant from a culture of dermal papilla fibroblasts, which is said to increase hair growth in the rat. U.S. Patent No. 5,358,714 to Green proposes the use of diacylglycerol activators of protein kinase C in order to increase or maintain hair growth in mammals, while U.S. Patent No. 5,068,315 to Buultjens et al. proposes the application of purified hair growth regulating peptides (HGRP) to stimulate hair growth. It has also been suggested that retinoids, substituted pyrimidines, and immunosuppressants be used as possible treatments for hair loss, although methods utilizing these compounds have not been entirely successful in producing a reliable and safe method of inducing hair growth. See G. Bazzano et al., *J. Invest. Dermatol.* 101 (1 Supplement), 138S-142S (1993); H. Jiang et al., *J. Invest. Dermatol.* 104(4), 523-525 (1995).

In recent years, the topical application of minoxidil has been a widely-used method for treating androgenic alopecia. See A.R. Zapacosta, *N. Eng. J. Med.* 303, 1480-81 (1980). U.S. Patent No. 4,139,619 to Chidsey, proposes a topical composition of minoxidil and related iminopyrimidines to stimulate the conversion of vellus hair to terminal hair and increase the rate of

-3-

growth of terminal hair. However, despite its popularity, minoxidil has not performed in a completely satisfactory fashion in promoting hair growth in all target populations.

5                    Summary of the Invention

A first aspect of the present invention is a method of treating alopecia in a subject in need thereof. The method comprises topically applying to the skin of the subject an estrogen receptor antagonist or a  
10 pharmaceutically acceptable salt thereof (an "active compound") in an amount sufficient to treat alopecia.

A more general aspect of the present invention is a method of promoting hair growth in a subject in need thereof. The method comprises topically applying to the  
15 skin of the subject an active compound as given above in an amount sufficient to promote hair growth.

A second aspect of the present invention is a topical pharmaceutical formulation comprising an active compound as given above in an amount effective to treat  
20 alopecia or promote hair growth, in a pharmaceutically acceptable topical carrier.

A third aspect of the present invention is the use of an active compound as given above for the preparation of a medicament useful for carrying out a  
25 method as given above.

Brief Description of the Drawings

**Figure 1A** is a photomicrograph showing the immunohistochemical localization of estrogen receptors in telogen hair follicle tissue from mouse skin, where  
30 immunohistochemical staining was conducted with hematoxylin counterstaining.

**Figure 1B** is photomicrograph showing the immunohistochemical localization of estrogen receptors in telogen hair follicle tissue from mouse skin, where

-4-

immunohistochemical staining was conducted without hematoxylin counterstaining.

Figure 1C is photomicrograph showing the immunohistochemical localization of estrogen receptors in early-anagen hair follicle tissue from mouse skin, where immunohistochemical staining was conducted with hematoxylin counterstaining.

Figure 1D is photomicrograph showing the immunohistochemical localization of estrogen receptors in early-anagen hair follicle tissue from mouse skin, where immunohistochemical staining was conducted without counterstaining.

Figure 2 is a graph illustrating that the estrogen receptor antagonist ICI 182,780 causes hair growth during the second telogen phase in mice. Closed triangles represent mice treated with 10 nmol ICI 182,780 in 200 $\mu$ L acetone applied topically to an area of clipped hair. Open circles represent control mice treated with 200  $\mu$ L acetone alone.

Figure 3A is a photograph of a mouse treated with 200  $\mu$ L of acetone applied topically to an area of clipped hair twice a week.

Figure 3B is a photograph of a mouse treated with 10 nmol ICI 182,780 in 200 $\mu$ L acetone applied topically to an area of clipped hair twice a week.

Figure 4A is a photomicrograph of skin tissue from a six-week-old mouse treated with 200  $\mu$ L of acetone applied topically to an area of clipped hair twice a week until seven weeks of age. The tissue was stained with hematoxylin/eosin.

Figure 4B is a photomicrograph of skin tissue from a six-week-old mouse treated with 10 nmol ICI 182,780 in 200 $\mu$ L acetone applied topically to an area of clipped hair twice a week until seven weeks of age. The tissue was stained with hematoxylin/eosin.

Figure 4C is a photomicrograph of skin tissue from a six-week-old mouse treated with 200  $\mu$ L of acetone



applied topically to an area of clipped hair twice a week until eight weeks of age. The tissue was stained with hematoxylin/eosin.

Figure 4D is a photomicrograph of skin tissue from a six-week-old mouse treated with 10 nmol ICI 182,780 in 200  $\mu$ L acetone applied topically to an area of clipped hair until eight weeks of age. The tissue was stained with hematoxylin/eosin.

Figure 5 is a graph illustrating that topical  $17\beta$ -estradiol blocks hair regrowth in mice. Six week old mice (5 mice per group) were clipped and treated with topical applications of either 10 nmol  $17\beta$ -estradiol in 200  $\mu$ L acetone (closed circles) or acetone alone (open circles), twice a week, up to sixteen weeks of age.

Figure 6A is a photomicrograph of skin tissue from a mouse, where 200  $\mu$ L of acetone was applied topically to shaved skin twice a week for three weeks. Tissue is stained with hematoxylin/eosin.

Figure 6B is a photomicrograph of skin tissue from a mouse, where 10 nmol  $17\beta$ -estradiol in 200  $\mu$ L acetone was applied topically to shaved skin twice a week for three weeks. Tissue is stained with hematoxylin/eosin.

Figure 6C is a photomicrograph of skin tissue from a mouse, where 200  $\mu$ L of acetone was applied topically to shaved skin twice a week for five weeks. Tissue is stained with hematoxylin/eosin.

Figure 6D is a photomicrograph of skin tissue from a mouse, where 10 nmol  $17\beta$ -estradiol in 200  $\mu$ L acetone was applied topically to shaved skin twice a week for five weeks. Tissue is stained with hematoxylin/eosin.

Figure 6E is a photomicrograph of skin tissue from a mouse, where 200  $\mu$ L of acetone was applied topically to shaved skin twice a week for seven weeks. Tissue is stained with hematoxylin/eosin.

-6-

**Figure 6F** is a photomicrograph of skin tissue from a mouse, where 10 nmol 17 $\beta$ -estradiol in 200  $\mu$ L acetone was applied topically to shaved skin twice a week for seven weeks. Tissue is stained with  
5 hematoxylin/eosin.

#### Detailed Description of the Invention

The method of the present invention is useful in the treatment of alopecia in mammals, and as such may be used to promote, increase, or assist in the growth of  
10 hair. Subjects may be male or female. As used herein, the term alopecia refers to the complete absence of hair in skin which typically exhibits hair growth, as well as a loss or diminution in the amount of hair. Multiple types and causes of alopecia are recognized in humans,  
15 including male pattern baldness, chemotherapy induced hair loss, congenital alopecia, and alopecia areata. As used herein, the term 'treating alopecia' refers to both the treatment of skin with a total absence of hair growth as well as treatment of skin having reduced or patchy  
20 hair growth.

The present invention is concerned primarily with the treatment of human subjects but may also be employed for the treatment of other mammalian subjects, such as dogs, cats, and sheep, for veterinary purposes  
25 (e.g., in the treatment of hair loss due to mange or other causes, or for enhancing wool or pelt production).

Hair, or pili, are fine threadlike appendages of the skin which normally cover the entire body (with the exception of the palms of the hands and soles of the  
30 feet, and the flexor surfaces of joints). A hair comprises a root embedded in the hair follicle and a free portion (the stem or shaft). As used herein, hair refers to mature hair as well as the soft, downy hair known as vellus hair.

35 The hair bulb, or follicle, is a compact structure located in the dermis layer of the skin and is

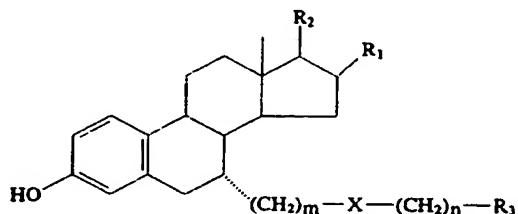
- 7 -

composed of three main cellular groups. The first comprises a compact group of fibroblasts known as the dermal papilla which includes a capillary system. The second group comprises germinative epithelial cells of the hair bulb which proliferate and differentiate to give rise to the mature hair shaft. The third group of fibroblasts exists around the outside of the bulb in the connective tissue sheath.

The present invention is, in general, carried out with estrogen receptor antagonists. The term "antagonist," as used herein, refers to compounds that act as complete or partial inhibitors of the activity of a biological substance by specifically binding to the receptor thereof. Numerous estrogen receptor antagonists are known in the art. It is specifically intended that both full and partial estrogen receptor antagonists are embraced by the scope of the invention, although full estrogen receptor antagonists are preferred.

Estrogen receptor antagonists may be steroids or non-steroid compounds. In one preferred embodiment of the invention, the estrogen receptor antagonist is a steroid. Particularly preferred are  $7\alpha$ -alkylamide analogs of estradiol,  $11\beta$ -alkylamide analogs of estradiol and  $16\alpha$ -halo- $17\beta$ -estradiols that have pure antiestrogenic activity.

In a preferred embodiment of the invention, the estrogen receptor antagonist is a compound of Formula I:



wherein m is from 1 to 15;  
n is from 1 to 5;

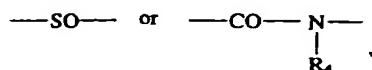
- 8 -

R<sub>1</sub> is hydrogen, lower alkyl, or halogen;

R<sub>2</sub> is oxygen or hydroxyl;

R<sub>1</sub> is lower alkyl or haloalkyl; and

X is



5                    wherein R<sub>a</sub> is loweralkyl.

Estrogen receptor antagonists suitable for use in the present invention include, but are not limited to, 7 $\alpha$ -[9-(4,4,5,5,5,-pentafluoropentylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17 $\beta$ -diol (also known as ICI 182,780), 10 N-n-butyl-N-methyl-11-(3,17 $\beta$ -dihydroxyestra-1,3,5(10)-triene-7 $\alpha$ -yl)undecanamide (also known as ICI 164,384), 11 $\beta$ -[4-[2-(dimethylaminoethoxy)phenyl]-estradiol (also known as RU 39411), N-methyl-N-isopropyl-(3,17 $\beta$ -dihydroxy-estra-1,2,5(10)-trien-11- $\beta$ -yl)-undecanamide (RU 15 51625), its 17 $\alpha$ -ethynyl derivative (RU 53637), [6-hydroxy-2(4-hydroxyphenyl)benzo[b]thien-3-yl] [4-[3-(1-pyrrolidin-yl)ethoxy]phenyl]methanone (LY117018), tamoxifen, clomiphene, keoxifene, 3-[4-(1,2-Diphenylbut-1-enyl)phenyl]acrylic acid, and the like, including the 20 active metabolites of these compounds. These compounds may be made in accordance with known procedures which will be apparent to those skilled in the art. See, e.g., A.E. Wakeling et al., *Canc. Res.* **51**, 3867-3873 (1991); D. Poirier et al., *J. Med. Chem.* **37**, 1115-1125 (1994); J. 25 Bowler et al., *Steroids* **54**, 71-79 (1989); C. Levesque et al., *J. Med. Chem.* **34**, 1624-1630 (1991); A. Claussner et al., *J. Steroid. Biochem. Mol. Biol.* **41**, 609-614 (1992).

The active compounds described herein can, as noted above, be prepared in the form of their pharmaceutically acceptable salts. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Examples of such

salts include (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids  
5 such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid,  
10 methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; (b) salts formed from elemental anions such as chlorine, bromine, and iodine; and (c) salts derived from bases, such as ammonium salts; alkali metal salts such as  
15 those of sodium and potassium; alkaline earth metal salts such as those of calcium and magnesium; and salts with organic bases such as dicyclohexylamine and N-methyl-D-glucamine.

The dosage of the active compound is not  
20 particularly critical so long as it achieves the desired effect. Actual dosage will depend on factors such as the particular pharmaceutical carrier, the particular skin region and condition being treated, and the general health and condition of the subject. In one embodiment  
25 of the invention, the active compound is topically administered to the affected skin of a subject in an amount sufficient to achieve a dose of at least .01 nanomoles (nmol), .1 nmol, or 1 nmol per 2 cm by 4.5 cm skin surface area, up to a dose of 100 nmol, 1,000 nmol,  
30 or 10,000 nmol or more per 2 cm by 4.5 cm skin surface area.

Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose  
35 administrations. The dose may be a single unit dose, which may, for example, be administered several times a week or from 1 to 3 times a day. Treatments may continue

-10-

week to week on a chronic basis as necessary (i.e., the active agent can be administered chronically). Administration of the active compounds may be carried out therapeutically or prophylactically, but preferably the compounds are administered therapeutically, either before substantial hair loss has occurred, or at a time when such hair loss has just begun.

Pharmaceutical compositions for use in the present method include those suitable for topical administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art. The most suitable route of administration in any given case may depend upon the anatomic location of the alopecia in the subject, the nature and severity of the condition being treated, and the particular active compound which is being used.

Pharmaceutical compositions useful in the present methods are preferably applied topically at the site of the affected skin. In alternative embodiments of the invention, pharmaceutical compositions useful in the present invention may be administered to a subject orally, parenterally (e.g., subcutaneously, intravenously, or intramuscularly), or transdermally (e.g., at a site other than the affected area to achieve systemic administration).

In a further aspect of the present invention, estrogen receptor antagonists may be used alone or in combination with one or more anti-alopecics for the prophylaxis or treatment of alopecia.

In the manufacture of a medicament according to the invention (a "formulation"), active agents or the physiologically acceptable salts thereof (the "active compound") are typically admixed with, inter alia, an acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the patient. The carrier may be a solid

-11-

or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, an aliquot of ointment which may contain from 0.001% to 99% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention (e.g., the formulation may contain one or more additional anti-alopecia agents as noted above), which formulations may be prepared by any of the well known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory therapeutic ingredients.

Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include vaseline, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Additionally, the compounds of the present invention may be administered by a liposome delivery system, as detailed in U.S. Patent No. 4,828,837 to Uster et al.

The compounds useful in the method of the present invention may be applied the surface of the skin at any anatomical location in need of treatment on the subject, including the scalp, pubis, face, chest, and legs. Administration of the compounds of the present invention onto the scalp of the subject is a preferred embodiment of the invention.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a

-12-

suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or molding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets may be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder. Formulations for oral administration may optionally include enteric coatings known in the art to prevent degradation of the formulation in the stomach and provide release of the drug in the small intestine.

Formulations of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the active compound, which preparations are preferably isotonic with the blood of the intended recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The formulations may be presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from



-13-

sterile powders, granules and tablets of the kind previously described.

Formulations suitable for transdermal administration (either at the site in need of treatment or at another site to achieve systemic administration) may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration may also be delivered by electrophoresis or iontophoresis (see, e.g., *Pharmaceutical Research* 3, 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound.

Although applicants do not wish to be bound to any particular theory of the instant invention, it appears that estrogen receptor antagonists cause telogen-phase hair follicles (resting follicles) to enter the anagen phase (active follicle) cycle, which results in the production of hair fiber and hair growth. Normally, approximately 90% of scalp hairs are in the anagen phase, with less than 1% in an intermediate catogen phase and the rest in telogen. With the onset of patterned baldness, however, a successively greater proportion of hairs are in telogen, while correspondingly fewer are found in the anagen phase.

The following examples are provided to more fully illustrate the present invention and should not be construed as limiting thereof. As used in the following examples, 17- $\beta$  Estradiol was purchased from Sigma Chemical Company (St. Louis, Missouri, USA); ICI 182,780 was a gift from Zeneca Pharmaceuticals (Cheshire, England); link antibody, label antibody and 3,3'-diaminobenzidine tetrahydrochloride/Super Sensitive Multi-link Immunostaining Kit were purchased from Biogenex Laboratorie (San Ramon, California, USA); 10X Automation Buffer was purchased from Biomeda Corporation, (Foster City, California, USA).

-14-

## EXAMPLE 1

Animals and Estrogen or  
Estrogen Receptor Antagonist Treatment

Female CD-1 mice, 4, 5, or 9 weeks of age, were  
5 purchased from Charles River Laboratories, Raleigh, NC.  
Mice were kept in an animal facility at least 1 week  
prior to use and were fed rodent chow (Agway Food,  
Granville Milling and Co., Creedmoor, NC) and water *ad*  
*libitum*. The mice were kept on corn cob bedding and  
10 placed on a 12 hour light/dark cycle. Female CD-1 mice  
were clipped on the dorsal region (approximately 4 x 2.5  
cm area) with electric clippers. The mice were then  
treated twice weekly on the clipped dorsal surface with  
either (a) 10 nmol 17- $\beta$ -estradiol in 200  $\mu$ l acetone or  
15 (b) acetone alone. For the studies with the estrogen  
receptor antagonist, ICI 182,780 was dissolved in acetone  
and 200  $\mu$ l was topically applied twice weekly.

## EXAMPLE 2

Localization of estrogen receptor in mouse skin

20 Seven-week-old female CD-1 mice were killed by  
cervical dislocation and the dorsal skin area was  
excised. The skins were fixed for 24 hours in a cold 10%  
neutral buffered formalin, then changed to cold 70%  
ethanol and processed and embedded in paraffin. Tissue  
25 sections were cut at 5  $\mu$ m and placed on SuperFrost Plus  
slides for immunohistochemistry. In order to localize  
the estrogen reception in skin, the paraffin sections  
were deparaffinized by two changes of xylene and  
rehydrated in a graded series of ethanol (100, 95 and  
30 70%) followed by 1x automation buffer. The skin sections  
were placed in 3% H<sub>2</sub>O<sub>2</sub> for 10 min to quench the endogenous  
peroxidase activity and then washed with 1x automation  
buffer. The sections were treated with fresh trypsin of  
0.15 mg/ml in 1x automation buffer for 4 min at room  
35 temperature followed by 1x automation buffer twice and  
incubated with fresh DNAase of 0.25 mg/ml in 1x

-15-

automation buffer for 3 min at room temperature followed by 1x automation buffer twice. The sections were blocked with 10% normal goat serum in 1x automation buffer for 15 min at room temperature. After blocking, the excessive  
5 solution was drained, and a prediluted primary antibody (Abbott ER-ICA monoclonal antibody, Abbott Laboratories, North Chicago, IL) was applied and incubated overnight at 4°C. Slides were washed in 1x automation buffer for 5 min twice and a secondary antibody (biotinylated goat  
10 anti-rat IgG, Boehringer Mannheim Corporation, Indianapolis, IN) at a dilution of 1:50 in 1x automation buffer was applied for 60 min at room temperature. After washing with 1x automation buffer for 5 min twice, the sections were incubated with peroxidase (HRP)-conjugated  
15 streptavidin (1:20 dilution, BioGenex, San Ramon, FL) for 30 min at room temperature and washed with 1x automation buffer for 5 min and 0.05M Tris-HCl (pH 7.5) for 5 min. The sections were then incubated with DAB (prepared according to the manufacturer's instruction) for 10 min  
20 in a dark box. Slides were rinsed with 0.05M Tris-HCl buffer twice and counterstained in hematoxylin for 5 seconds followed by rinsing in distilled water five times. Finally, the sections were dehydrated in a graded series of ethanol and xylene, and then slides were  
25 permanently mounted with Permount.

Immunohistochemical staining for the estrogen receptor in mouse skin revealed intense and specific staining of the nuclei of cells with the dermal papilla of a telogen follicle, as shown in the counterstained  
30 sample **Figure 1A**. Non-counterstained samples (**Figure 1B**) are provided to better demonstrate the areas and levels of estrogen receptor expression. Estrogen receptor expression within most telogen dermal papilla demonstrated a polarity, as nuclei of cells within the  
35 lower half of the dermal papilli stained intensely, while very little staining was observed in the upper half of dermal papilla. In addition, the expression of the

-16-

estrogen receptor was hair cycle dependent as there was a weaker staining of the dermal papilla of mid to late catagen or anagen follicles (Figures 1C and 1D), and no detectable staining in dermal papilla of mid to late anagen follicles (data not shown). Very light estrogen receptor staining was observed in cells of the outer root sheath in the isthmus of the telogen follicle as well as in some nuclei of dermal fibroblasts.

### EXAMPLE 3

#### Estrogen receptor antagonist ICI 182,780 induces hair growth

Mice were treated with the estrogen receptor antagonist ICI 182,780 as provided in Example 1. Twice-weekly treatment of clipped dorsal skin was begun when the mice were 6 weeks of age, with either (a) 10 nmol ICI 182,780 in acetone or (b) acetone alone. As shown in Figure 2 and Figure 3, by 9 weeks of age, 60% of the ICI 182,780 treated mice demonstrated full hair regrowth while no hair growth was observed in the acetone treated mice. By 10 weeks of age all of the ICI 182,780 treated mice developed a full coat of hair while only 40% of the acetone-treated mice demonstrated full hair regrowth.

These data indicate that ICI 182,780 caused the telogen follicle to enter anagen during what should have been the second synchronized telogen phase. To confirm this result, skin was collected and prepared for histological analysis at 7, 8, 9, 10 and 11 weeks. At 7 weeks of age the ICI 182,780 treated mice demonstrated follicles that were already in early to mid anagen, while the follicles of the acetone-treated mice were synchronized in second telogen (Figures 4A and 4B). By eight weeks of age the follicles of the ICI 182,780 treated mice demonstrated follicles that were in mid to late anagen while the hair follicles in acetone-treated mice were in telogen (Figures 4C and 4D). These results indicate that the estrogen receptor antagonist ICI

-17-

182,780 initiates the transition of a telogen follicle into anagen. At eleven weeks of age, the follicles of mice treated with ICI 182,780 entered telogen, while control mice were in mid to late anagen (data not shown),  
5 indicating that ICI 182,780 does not prolong anagen.

#### EXAMPLE 4

##### 17- $\beta$ estradiol inhibits hair growth

In order to determine if 17- $\beta$ -estradiol could influence hair growth, six week old CD-1 female mice were  
10 clipped on the dorsal surface with electric clippers and then treated twice weekly from the 6th week of age to the 16th week of age with topical applications of either (a) 10 nmol 17- $\beta$ -estradiol or (b) acetone vehicle alone. Treatment with 17- $\beta$ -estradiol had a potent inhibitory  
15 effect on dorsal hair regrowth. As shown in Fig. 5, 100% of the acetone-treated mice demonstrated full hair regrowth by 13 weeks of age. In contrast, mice treated with 17- $\beta$ -estradiol did not demonstrate any full hair regrowth by 16 weeks of age. Some of the 17- $\beta$ -estradiol-  
20 treated mice did demonstrate a partial patchy hair regrowth that involved less than 10% of the total clipped area. At 12 weeks of age, 20% of the 17- $\beta$ -estradiol-treated mice demonstrated partial patchy hair regrowth and by 15 weeks 40% of the 17- $\beta$ -estradiol-treated mice  
25 demonstrated such partial patchy hair regrowth (data not shown).

The above results demonstrate that 17- $\beta$ -estradiol treatment just before and during the 2nd telogen phase potently blocked hair growth. In order to  
30 determine if 17- $\beta$ -estradiol could block hair growth in pre-existing anagen follicles, 10 week old mice in the anagen phase of the hair cycle were treated twice weekly with 17- $\beta$ -estradiol applied topically to clipped dorsal skin. Within two weeks it was apparent that the 17- $\beta$ -  
35 estradiol-treated mice were no longer growing hair while all the acetone mice developed a full coat of hair (data

-18-

not shown). Treatment with 17- $\beta$ -estradiol was continued for 10 weeks and during this period no hair growth was apparent. The results of this experiment demonstrate that 17- $\beta$ -estradiol can also block hair growth in mouse skin treated during the 3rd synchronous anagen phase.

#### EXAMPLE 6

##### Effect of 17- $\beta$ -estradiol on the hair follicle cycle

To determine the effect of 17- $\beta$ -estradiol on the hair follicle cycle, mice were treated with 17- $\beta$ -estradiol or acetone alone twice weekly from the 4th week of age to the 15th week of age. Every week, histological analysis of the skin from 3 mice from each group was conducted to determine the phase of the hair cycle. Representative skin histology sections from acetone- and 17- $\beta$ -estradiol-treated mice at 7, 9, and 11 weeks of age are shown in **Figures 6A-6F**. As expected, at 7 weeks of age all follicles were in telogen in both the 17- $\beta$ -estradiol and the acetone-treated mice. By 9 weeks of age, the hair follicles of the acetone control mice entered anagen while the hair follicles of the 17- $\beta$ -estradiol mice remained in telogen. By 11 weeks of age, the hair follicles of the acetone treated mice were all in mid to late anagen while the hair follicles of the 17- $\beta$ -estradiol-treated mice remained in telogen. At the termination of the experiment, when the mice were 15 weeks of age, the hair follicles of the 17- $\beta$ -estradiol-treated mice were still arrested in telogen (data not shown). Collectively these data demonstrate that the topical application of 17- $\beta$ -estradiol prevents hair re-growth by arresting the hair follicle in the telogen phase of the hair cycle.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

-19-

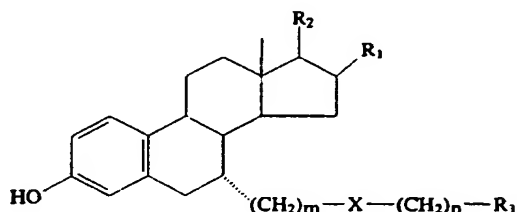
## THAT WHICH IS CLAIMED IS:

1. A method of treating alopecia in a subject in need thereof comprising topically applying to the skin of said subject an estrogen receptor antagonist or a pharmaceutically acceptable salt thereof in an amount  
5 sufficient to treat alopecia.
2. A method according to claim 1, wherein said estrogen receptor antagonist is a steroid.
3. A method according to claim 1, wherein said subject is a female subject.
- 10 4. A method according to claim 1, wherein said subject is a male subject.
5. A method according to claim 1, wherein said estrogen receptor antagonist is applied to the scalp of a subject.
- 15 6. A method according to claim 1, wherein said skin contains telogen-phase hair follicles.
7. A method according to claim 1, wherein said estrogen receptor antagonist is selected from the group consisting of  $7\alpha$ -alkylamide analogs and  $11\beta$ -  
20 alkylamide analogs of estradiol.
8. A method according to claim 1, wherein said estrogen receptor antagonist is selected from the group consisting of  $7\alpha$ -[9-(4,4,5,5,5,-pentafluoropentylsulfinyl)nonyl]estra-1,3,5(10)-triene-  
25 3,17 $\beta$ -diol, N-n-butyl-N-methyl-11-(3,17 $\beta$ -dihydroxyestra-1,3,5(10)-triene-7 $\alpha$ -yl)undecanamide, 11 $\beta$ -[4-[2-(dimethylaminoethoxy)phenyl]-estradiol, N-methyl-N-isopropyl-(3,17 $\beta$ -dihydroxy-estra-1,2,5(10)-trien-11- $\beta$ -yl)-undecanamide, [6-hydroxy-2(4-

- 20 -

hydroxyphenyl)benzo[b]thien-3-yl] [4-[3-(1-pyrrolidin-yl)ethoxy]phenyl]methanone, 3-[4-(1,2-Diphenylbut-1-enyl)phenyl]acrylic acid, tamoxifen, clomiphene, and keoxifene.

5                    9.    A method according to claim 1, wherein  
said estrogen receptor antagonist is a compound of  
Formula I:



wherein m is from 1 to 15;

```
n is from 1 to 5;
```

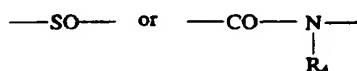
10

R<sub>1</sub> is hydrogen, lower alkyl, or halogen;

R<sub>2</sub> is oxygen or hydroxyl;

R<sub>3</sub> is lower alkyl or haloalkyl; and

X is



wherein R<sub>4</sub> is loweralkyl,

15 or a pharmaceutically acceptable salt thereof.

10. A method according to claim 9, wherein

m is 9;

n is 3;

R<sub>1</sub> is hydrogen;

20

R<sub>2</sub> is hydroxyl;

R<sub>3</sub> is haloalkyl; and

X is -SO-.

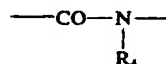


-21-

11. A method according to claim 10, wherein  
R<sub>3</sub> is -CF<sub>2</sub>CF<sub>3</sub>.

12. A method according to claim 9, wherein  
m is 10;  
n is 3;  
R<sub>1</sub> is hydrogen;  
R<sub>2</sub> is hydroxyl;  
R<sub>3</sub> is lower alkyl;  
X is

10



, and

R<sub>4</sub> is methyl.

13. A method of promoting hair growth in a  
subject in need thereof comprising topically applying to  
the skin of said subject a compound selected from the  
group consisting of estrogen receptor antagonists and  
pharmaceutically acceptable salts thereof in an amount  
sufficient to promote hair growth.

14. A method according to claim 13, wherein  
said subject is afflicted with hair loss, and said  
compound is administered in an amount effective to treat  
hair loss.

15. A topical pharmaceutical formulation  
comprising an estrogen receptor antagonist or a  
pharmaceutically acceptable salt thereof in an amount  
effective to treat alopecia in a pharmaceutically  
acceptable topical carrier.

-22-

16. A topical pharmaceutical formulation of claim 15, wherein said estrogen receptor antagonist is a steroid.

17. A topical pharmaceutical formulation of  
5 claim 15, wherein said estrogen receptor antagonist is  
7 $\alpha$ -[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]estra-  
1,3,5(10)-triene-3,17 $\beta$ -diol.

18. A topical pharmaceutical formulation of claim 15, wherein said formulation is a gel.

10 19. A topical pharmaceutical formulation of claim 15, wherein said formulation is a spray.

1/15



FIG. 1A.

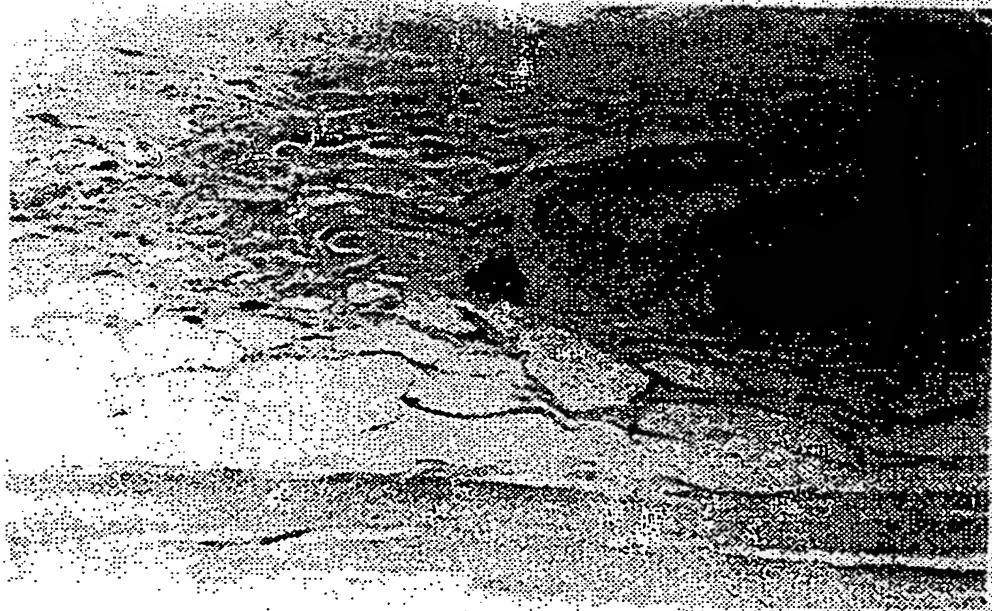


FIG. 1B.

SUBSTITUTE SHEET (RULE 26)

2/15

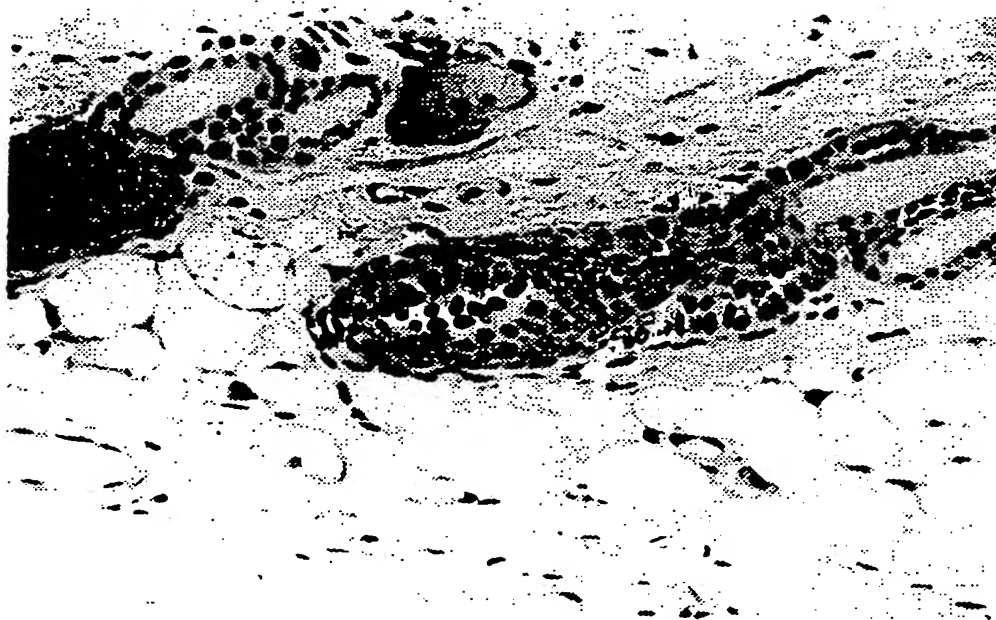


FIG. IC.

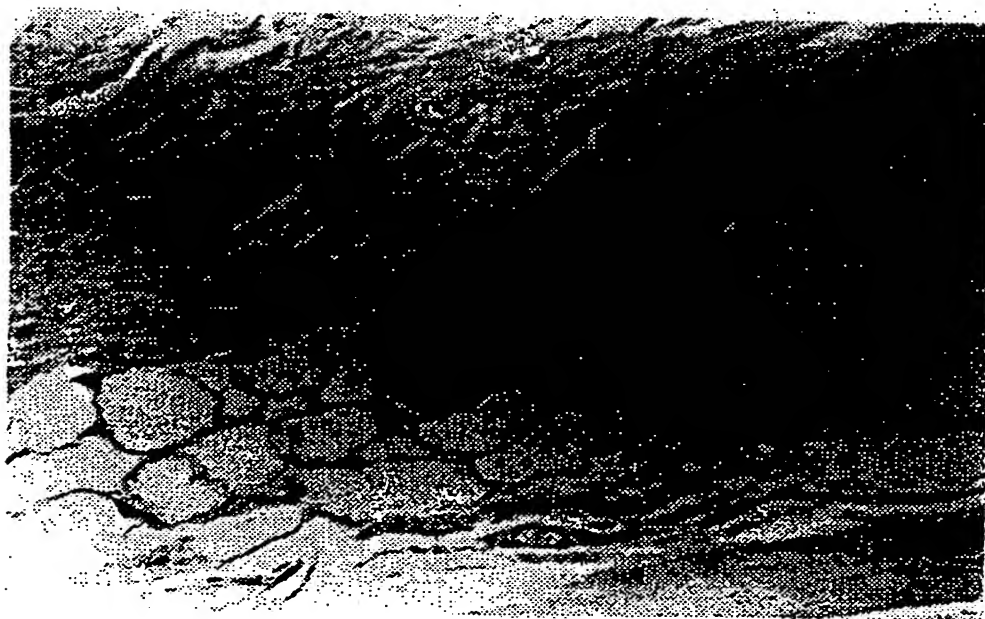
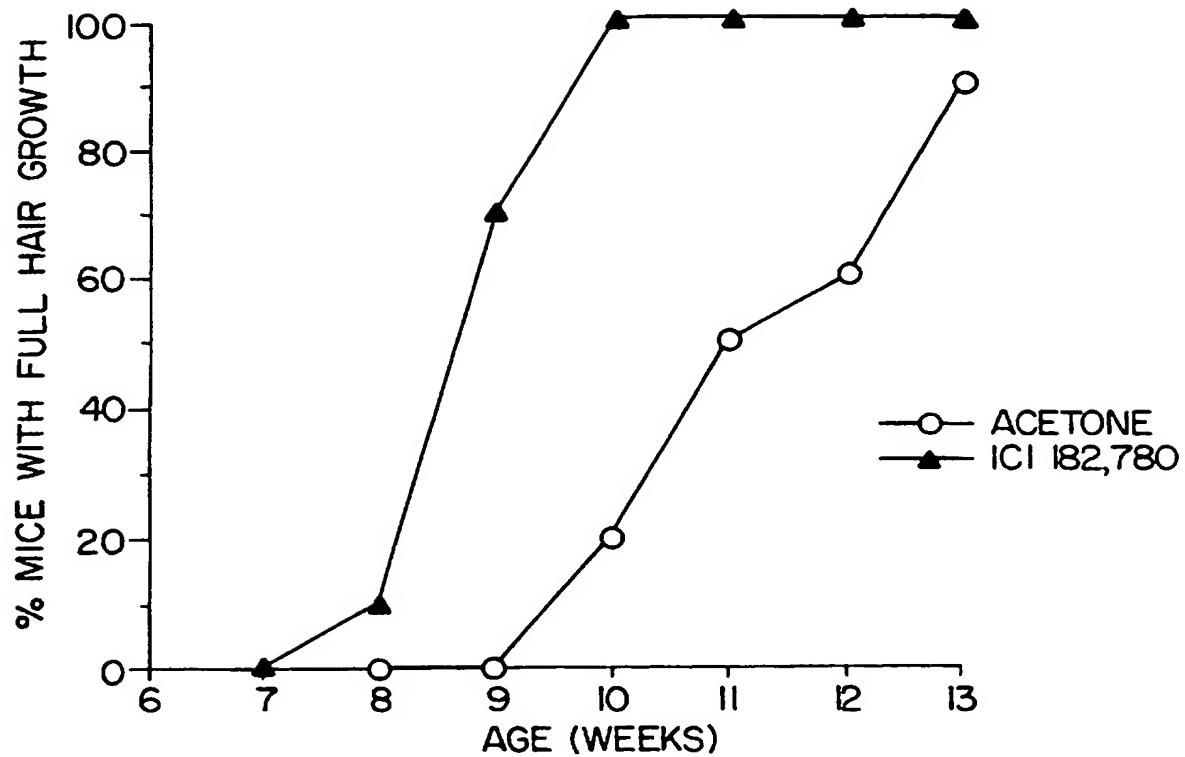


FIG. ID.

SUBSTITUTE SHEET (RULE 26)

3/15

FIG. 2.

SUBSTITUTE SHEET (RULE 26)

4/15

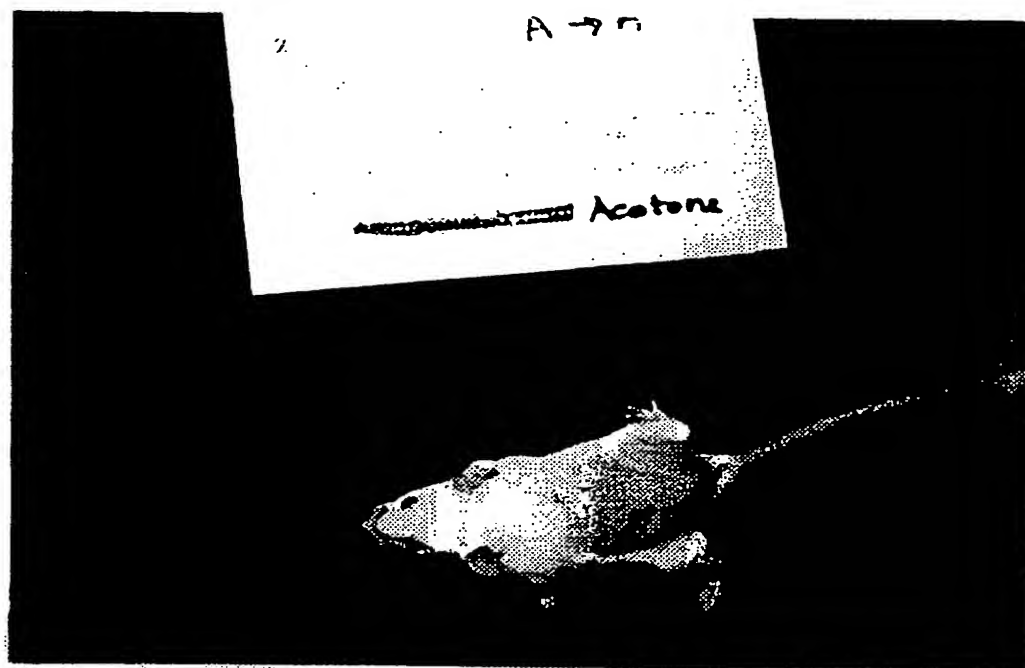


FIG. 3A.

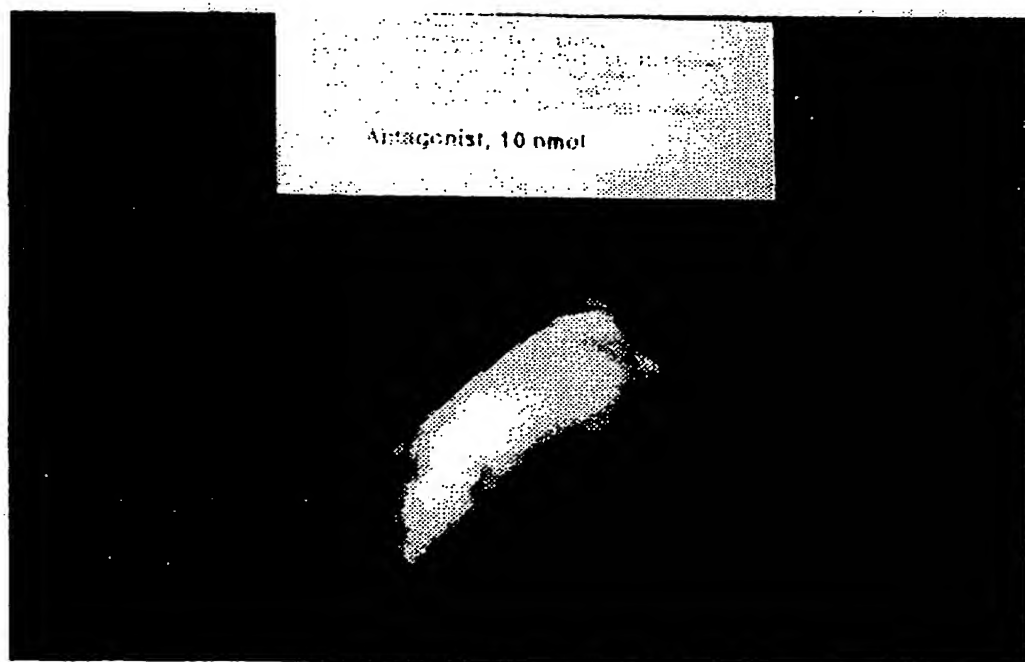


FIG. 3B.

SUBSTITUTE SHEET (RULE 26)

5/15



FIG. 4A.

SUBSTITUTE SHEET (RULE 26)

6/15



FIG. 4B.

SUBSTITUTE SHEET (RULE 26)



7/15

FIG. 4C.

SUBSTITUTE SHEET (RULE 26)

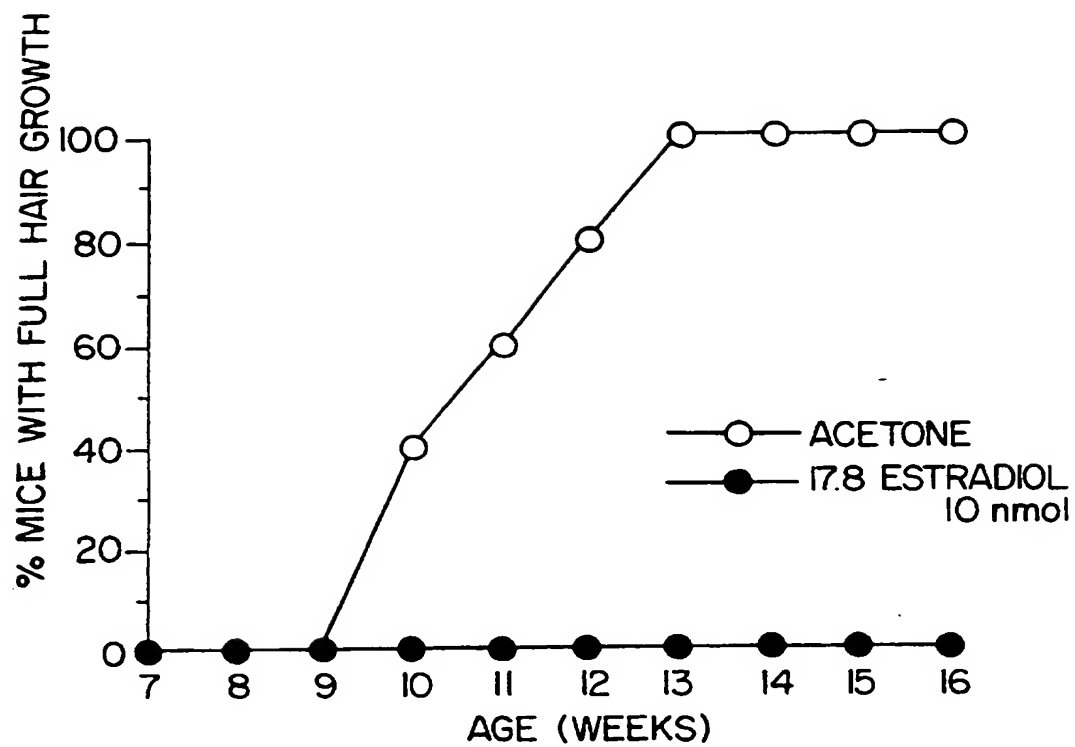
8/15



FIG. 4D.

SUBSTITUTE SHEET (RULE 26)

9/15

FIG. 5.

10/15



FIG. 6A.

SUBSTITUTE SHEET (RULE 26)

11/15



FIG. 6B.

SUBSTITUTE SHEET (RULE 26)

12/15



FIG. 6C.

SUBSTITUTE SHEET (RULE 26)



13/15



FIG. 6D.

SUBSTITUTE SHEET (RULE 26)

14/15



FIG. 6E.

SUBSTITUTE SHEET (RULE 26)



15/15



FIG. 6F.

SUBSTITUTE SHEET (RULE 26)

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/02385

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 9/127

US CL : 514/178

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/178

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,395,842 (LABRIE et al.) 07 March 1995. See entire document.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A document defining the general state of the art which is not considered to be of particular relevance	*X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E earlier document published on or after the international filing date	*Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z document member of the same patent family
*O document referring to an oral disclosure, use, exhibition or other means	
*P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

05 JUNE 1997

Date of mailing of the international search report

11 JUL 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

ZOREH FAY

Facsimile No. (703) 305-3230

Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)\*